

稳定过表达smac 基因胃癌细胞株的建立及其化疗敏感性研究

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Establishment of Gastric Cancer Cell Line Stable Overexpressing smac Gene and Its Chemotherapeutic Sensitivity

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摘要 目的 探讨smac 基因过表达对胃癌细胞株化疗敏感性的影响。方法 采用脂质体将smac 基因真核表达载体pcDNA3. 1-smac 及空载体pcDNA3. 1 转染入胃癌细胞MKN245, G₄₁₈ 筛选获得抗性亚克隆细胞株, RT-PCR 和Western Blot 检测癌细胞smac 基因表达, 四甲基偶氮唑盐(MTT) 比色法、克隆形成实验检测丝裂霉素(MMC) 对癌细胞的生长抑制效率。结果 建立分别稳定表达smac 基因、新霉素抗性基因(neo) 的胃癌亚克隆细胞株MKN-45/ smac、MKN-45/ neo。RT-PCR 和Western Blot 证实MKN245/ smac 细胞的smac mRNA 及蛋白表达水平均显著高于MKN-45、MKN-45/ neo (P < 0. 01)。10μg/ ml MMC 作用24h 后, MKN245、MKN245/ neo 细胞生长抑制率分别为27. 85 %、28. 12 %, 而MKN-45/ smac 则高达43. 71 % (P < 0. 01); MKN-45、MKN-45/ neo 细胞株比较, MKN-45/ smac 的克隆形成能力分别降低了14. 07 % (P < 0. 01)、15. 13 % (P < 0. 01)。结论 稳定转染smac 基因使其在胃癌细胞株中过表达, 能显著提高癌细胞对MMC 的敏感性, 为改善胃癌化疗效果奠定了实验基础。

关键词: 胃癌 smac 基因 基因表达 细胞凋亡

Abstract: Objective To explore the effects on chemotherapeutic sensitivity of gastric cancer cell line by stable overexpression of smac gene. Methods Under the induction of liposome, the eukaryotic expression vector pcDNA3. 1-smac for smac gene and its control vector pcDNA3. 1 were transfected into gastric cancer cell line MKN-45. The subclone cell lines were obtained by persistent G₄₁₈ selection. smac gene expression of cancer cells were detected by RT-PCR and Western Blot methods. The growth inhibition effects of mitomycin (MMC) on cancer cells were also observed by tetrazolium bromide colorimetry and clone formation test. Results The subclone gastric cancer cell lines, stable expressing smac and neo gene respectively, were successfully selected, named as MKN-45/ smac and MKN-45/ neo. RT-PCR and Western Blot results demonstrated smac mRNA and protein levels of MKN-45/ smac cells were significantly higher than those of MKN-45 and MKN-45/ neo (P < 0. 01). After being treated with 10 μg/ ml MMC for 24h, the growth inhibition rates of MKN-45 and MKN-45/ neo were 27. 85 %, 28. 12 % respectively, with that of MKN-45/ smac cells being 43. 71 % (P < 0. 01). When compared with MKN-45 and MKN245/ neo cells, the clone formation abilities of MKN245/ smac were reduced by 14. 07 % (P < 0. 01), 15. 13 % (P < 0. 01) respectively. Conclusion Stable transfection of smac gene and its over-expression in gastric cancer cell line could significantly improve their chemotherapeutic sensitivities to MMC, which established an experimental basis for ameliorating chemotherapy of gastric cancer.

Key words: Gastric cancer smac gene Gene expression Apoptosis

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